Investigating the modulation effects of polyphenols on cancer gut microbiome and metabolome

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Introduction & Objectives

Human Colonic Model

Figure 1. The schematic of this study

Target metabolite analysis

Statistical analysis

The gut microbiome affects many aspects of human health including response to cancer treatments. Recent work has demonstrated that certain gut bacteria modulate response to immune checkpoint inhibitors[1] (ICIs), and, in mice, response to ICIs can be increased by increasing the abundance of probiotics[2]. Diet-based interventions hold promise for translating microbiome modification into a clinical setting; however, progress has unfortunately been slowed by a lack of evidence regarding the ways in which specific food products can affect microbes, particularly in complex communities such as the gut. Increasing the abundance of probiotics thus represents a promising approach to modulate the likelihood of response to ICIs in humans. As such, it is important to maintain a healthy gut microbiota and correct any dysbiosis as it serves important functions in maintaining the gut barrier's structural integrity, and enhance responses to certain cancer treatments[3,4]. As reported elsewhere, the density of probiotics can be influenced by dietary interventions such as fruits rich in polyphenols[5]. The objective of the project focuses on the use of novel *in vitro* human colonic model to simulate a colon environment without the interference from the host, to study the interaction between gut microbe and polyphenol-rich black raspberry (BRB) extract and characterization of its metabolites. This will provide insights on how dysbiosis in cancer patient might be corrected using food interventions and the roles played by the associated phenolic compounds. Additional metagenomic analysis that is currently underway will provide more information on how proportion of gut microbes respond to BRB supplementation.

Materials and Methods

Microbial experiments:

1. A customized human colonic model was established using automatic control modules, a series of bioreactors, and operated in anaerobic conditions at 37°C without light exposure.
2. Microbiome from lung cancer patient was extracted from fecal sample and cultured overnight in anaerobic chamber. 3 ml of bacteria culture was added to each vessels.
3. HCM was run for 6 weeks, with 2 weeks of pre-treatment, 2 weeks of treatment and 2 weeks of post treatment, samples were collected and analysed. 5ml/L of black raspberry extract was added to the feeding media as treatment. Samples were collected and stored in -80 ℃ for further analysis.
4. Gut microbial population and diversity were measured via 16s rRNA gene sequencing and transcriptomic analysis was performed to measure the changes in gene expression.

Mass spectrometry experiments:

1. Authentic metabolites standards were used to establish the targeted compound detection.
2. A Vanquish UHPLC Systems coupled with Q Exactive UHMR hybrid Qudrupole-Orbitrap MS System from ThermoFisher Scientific were used for metabolomics analysis.
3. Data were analyzed in Compound Discover software and MetaboAnalyst for statistical analysis.

Results

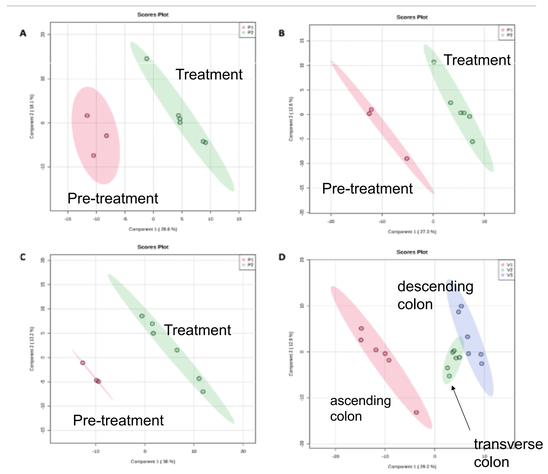

Figure 2. **A:** PLSDA plot of intracellular polar metabolite profile between pre-treatment and treatment phases in **A)** ascending colon; **B)** transverse colon; and **C)** descending colon. Clear separation in PLSDA plot means distinctly changed metabolic profile after BRB intervention. **D)** PLSDA plot of locational comparison in three colon sections during treatment phase.

Results (Cont.)

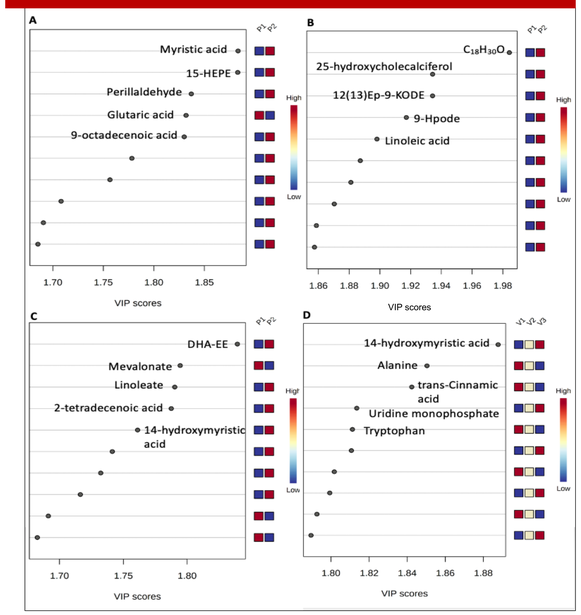

Figure 3. VIP score plot shown the top 5 metabolites that drive the distinct changes in metabolic profile in **A)** ascending colon; **B)** transverse colon; and **C)** descending colon during the pretreatment phase and treatment phase. **D** shown the location comparison analysis of the colon during treatment phase.

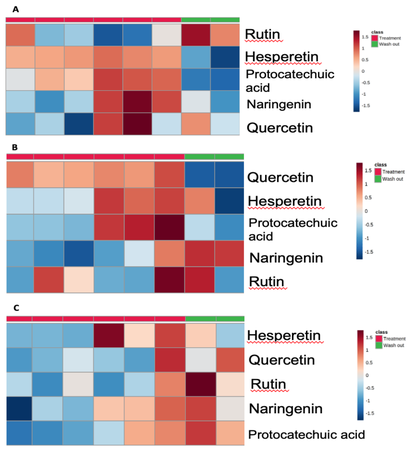

Figure 4. Heat map showing the changes in the amount of phenolic compounds found in colon between treatment and washout phase. **A)** ascending colon; **B)** transverse colon; and **C)** descending colon.

Results (Cont.)

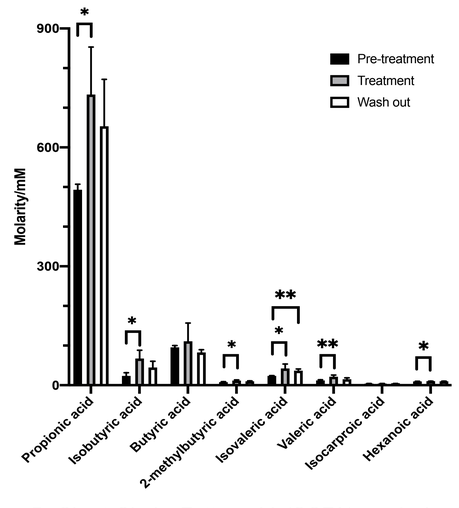

Figure 5. Short-Chain Fatty acid (SCFA) analysis during pre-treatment, treatment and post-treatment phases in ascending colon**.** Statistical analysis shown that propionic acid, isobutyric acid, 2-methylbutyric acid and hexanoic acid shown a significant increase in concentration between pre-treatment to treatment phase in asceding colon (t-test p <0.05). Isovaleric acid and valeric acid also shown a significant increase in their concentration (t-test p <0.01).

Summary & Future Directions

- 6 out of 8 SCFA metabolites were detected at higher relative abundance after BRB treatment, showing that there is a polyphenol-driven change in the metabolic profile of the microbiome.
- Additional metagenomic analysis is currently ongoing and will provide a better understanding of how different gut microbes respond to the addition of black raspberry extract.

References

1. Yi, M., Yu, S., Qin, S., Liu, Q., Xu, H., Zhao, W., Chu, Q., & Wu, K. (2018). Gut microbiome modulates efficacy of immune checkpoint inhibitors. *Journal of hematology & oncology*, *11*(1), 47. https://doi.org/10.1186/s13045-018-0592-6
2. Zhang, T., Li, Q., Cheng, L., Buch, H., & Zhang, F. (2019). Akkermansia muciniphila is a promising probiotic. *Microbial biotechnology*, *12*(6), 1109–1125. https://doi.org/10.1111/1751-7915.13410
3. Reunanen, J., Kainulainen, V., Huuskonen, L., Ottman, N., Belzer, C., Huhtinen, H., de Vos, W. M., & Satokari, R. (2015). Akkermansia muciniphila Adheres to Enterocytes and Strengthens the Integrity of the Epithelial Cell Layer. *Applied and environmental microbiology*, *81*(11), 3655–3662. https://doi.org/10.1128/AEM.04050-14
4. Matson, V., Fessler, J., Bao, R., Chongsuwat, T., Zha, Y., Alegre, M. L., Luke, J. J., & Gajewski, T. F. (2018). The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science (New York, N.Y.)*, *359*(6371), 104–108. https://doi.org/10.1126/science.aao3290
5. Anonye B. O. (2017). Commentary: Dietary Polyphenols Promote Growth of the Gut Bacterium *Akkermansia muciniphila* and Attenuate High-Fat Diet-Induced Metabolic Syndrome. *Frontiers in immunology*, *8*, 850. https://doi.org/10.3389/fimmu.2017.00850

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